INVESTIGATIONS ON NITROSAMINE REDUCTION IN BONELESS HAMS PROCESSED IN ELASTIC RUBBER NETTINGS

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ABSTRACT

N-Nitrosamines have been found in a wide variety of rubber-containing products. Since many types of boneless hams are processed in elastic nettings made with rubber, the potential exists for forming nitrosamines. In this paper we report that addition of excess nitrite to outer surface ham and netting samples results in a significant increase in N-nitrosodibenzylamine (NDBzA). Hams were then prepared with 100 ppm ingoing $NaNO_2$ and compared to a standard cure of 200 ppm $NaNO_2$. A highly significant reduction ($p < 0.01$) in NDBzA (31%) was noted in those made with the lower level of nitrite. In another series of experiments, hams were stored at either -2.2C or 2.2C, for up to six or 12 weeks, with the nettings on or off the ham. The overall results suggest that the level of NDBzA increased with netting contact time. Therefore it is advisable to remove the nettings after processing.

INTRODUCTION

Of the estimated $18 billion sales of packaged meats, including deli and nondeli items, $2.5 billion is comprised of cured ham and picnics (Anon 1993). The sales of these products are projected to increase due to consumer demand for lower fat, lower calorie, sensory appealing, and more convenient meat products. A large segment of these sales are boneless hams that are processed with elastic nettings, which are used to provide compression to help bind the meat together during smokehouse processing. These nettings contain rubber, and with it additives used in its formulation that have the potential for forming N-nitroso compounds, more commonly called nitrosamines. Nitrosamines comprise a class of compounds of which 90% or more of its members have exhibited carcinogenic activity in a wide range of animal

species, including primates (Preussmann and Stewart 1984; Bogovski and Bogovski 1981). This is why there is a concern about exposure to nitrosamines in foods. Nitrosamines were found to form from rubber vulcanization accelerators, compounds made from and decomposing to secondary amines (Ireland *et al.* 1980; Yeager *et al.* 1980). These additives help impart the desired physical characteristics to rubber. As a result, nitrosamines have been detected in a wide variety of rubber-containing products that include: tires, tubing, stoppers, toys, gloves, baby bottle nipples and pacifiers (Spiegelhalder and Preussmann 1982; Tricker *et al.* 1989). We also recently reported nitrosamines in rubber bands used for orthodontic purposes (Fiddler *et al.* 1992). Of all the reports of nitrosamines in rubber products, most of the concern was focused on the possible migration of nitrosamines from baby bottle nipples and pacifiers into drinks and formulas (Preussmann *et al.* 1982).

Sen *et al.* (1987) reported the finding of N-nitrosodibutylamine (NDBA) and N-nitrosodiethylamine in several cured pork products processed in elastic rubber nettings. Just as in the case of the migration of nitrosamines from rubber baby bottle nipples into food became a matter of concern, so did the association between hams and elastic rubber nettings. Unlike the nipples and pacifiers, there is the potential for additional nitrosamine formation due to nitrite in the ham product that is in direct contact with the components in the netting. This became more important when we recently found that both NDBA and N-nitrosodibenzylamine (NDBzA) and their precursor amines were present in the hams to a depth of 3.18 cm (Pensabene *et al.* 1995). As a result we investigated one pre- and one post-processing method that would help reduce the nitrosamine content of netted hams. The results are reported in this paper.

MATERIALS AND METHODS

Safety Note: Precaution should be exercised in the handling of nitrosamines, since they are potential carcinogens.

Nitrosation Potential Study

The 100.0 g of comminuted sample, from the outer surface of a ham that had previously been analyzed for nitrosamines, was mixed with an additional 1000 ppm of sodium nitrite. The sample was heated for 20 min in an oven set at 191C, cooled, then reanalyzed for nitrosamines. For the unused nettings, 0.5 g was extracted for 18 h in 0.2 N HCl. The netting was removed from the acid and 1000 ppm sodium nitrite (500 μg) added with stirring at room temperature over a period of 1 h. The reaction was

quenched with sulfamic acid, the acid extracted with dichloromethane (DCM), the DCM dried, concentrated, and then quantified by GC-TEA.

Ham Storage Study

This storage study was carried out in three parts. Initially, unused elastic rubber nettings from three different lots were analyzed for the presence of NDBzA. The lot containing the highest NDBzA level was chosen for use in the preparation of boneless hams. All hams were processed at a local meat processing facility specializing in the preparation of cured pork products. The finished product, with the netting still on the ham, was sent to ERRC immediately after processing. The hams were quartered lengthwise, then vacuum packaged and stored at -2.2C for 0, 4, 8, and 12 weeks. At the appropriate time interval, the ham was removed from storage, the netting removed, the outer 0.64 cm original surface (that in contact with the netting) removed and ground through a 16 mm plate. Residual sodium nitrite analysis was performed, and the remaining sample vacuum packaged and stored in a -20C freezer until analyzed for nitrosamines. The used netting was cut into small pieces and analyzed for nitrosamines as described previously (Pensabene *et al.* 1995). In the second experiment, unused nettings from three additional lots were again analyzed for NDBzA, and the lot containing the highest level chosen for processing. Boneless hams were processed at a local processor, then shipped immediately after processing to ERRC. The hams were prepared as described above, except that the storage times were 0, 2, 4 and 6 weeks, and the storage temperature was 2.2C. After storage, the hams were treated and analyzed as described above. Initially, only the outer 0.64 in. of ham was removed; however, when this experiment was repeated, the second 0.64 in. depth was removed from the ham in addition to the 0.64 in. outer surface. In the third experiment, the same protocol was used as described in part two, except this time, some of the nettings were removed at zero time in order to make a direct comparison with the samples stored with the netting on. All of the hams used in this part of the study were also analyzed for dibenzylamine (DBzA).

Ingoing Nitrite Study

Boneless hams were processed at a local meat processing plant in nettings from the same lot used in the first part of the ham storage study. Ingoing sodium nitrite levels were either 100 ppm or 200 ppm, with all other processing variables the same. After smokehouse processing, the hams were immediately sent to ERRC. Upon receipt, the nettings were removed, vacuum packaged, and stored at -20C till analyzed. A 0.64 cm center cross-sectional slice was also removed from the intact ham prior to removal of the outer

0.64 cm of the ham. Both samples were then ground through a 16 mm plate. Residual sodium nitrite was determined, then the ham samples vacuum packaged and stored at -20C until analyzed for nitrosamines.

Nitrosamine Analysis

The details for the isolation of nitrosamines from the ham samples, using a solid phase extraction procedure, and quantified by gas chromatography-Thermal Energy Analyzer (GC-TEA) have been published elsewhere (Pensabene and Fiddler 1994). The nitrosamine values have been corrected for the recovery of the 10 ppb internal nitrosamine standard (N-nitrosodi-propylamine, NDPA) in each individual sample. The minimum detectable level (signal:noise > 2) for NDBzA was 0.5 ppb. Nitrosamines in the nettings were determined by cutting the netting into small pieces, soaking them overnight in DCM, concentrating, then quantitating on the GC-TEA as described previously (Pensabene *et al.* 1995).

Amine Analysis

The procedure for the isolation and quantitation of dibenzylamine (DBzA) has been reported elsewhere (Pensabene *et al.* 1995).

Data Analysis

Data were analyzed by the General Linear Model and Means procedures of the Statistical Analysis System PC software (SAS Institute 1985). The results were interpreted according to methods of Snedecor and Cochran (1979) and Youden and Steiner (1975).

RESULTS AND DISCUSSION

An earlier study on the formation and penetration of NDBA and NDBzA into hams processed in elastic rubber nettings suggested that there was a potential to form additional nitrosamine, since significant amounts of the precursor amines, DBA and DBzA, were found on the ham surface after processing (Pensabene *et al.* 1995). To confirm this, samples were taken from the exterior surface of hams previously analyzed for NDBA and nitrosated. Four of 5 samples gave higher NDBA values after nitrosation; the mean value increased from 29.4 to 66.6 ppb. One of these samples gave an increase of 350%. Similar results were obtained from 6 hams containing NDBzA, with a mean increase of 70.8%, 104 to 177 ppb. The increase in nitrosamine formation is most likely due to the nitrosation of the amine that migrates into the product to a depth of 3.18 cm or more (Pensabene *et al.*

1995). Recent studies on the thermal decomposition of purified zinc dibenzyldithiocarbamate (Zn DBzDTC) suggest that DBzA, a contaminant in the technical or commercial grade accelerator, may have an important role in the formation of NDBzA in hams (Helmick and Fiddler 1994). Additionally, more DBzA may be formed from the decomposition of Zn DBzDTC. While the addition of 1000 ppm sodium nitrite used in this experiment may be considered extreme, it nevertheless illustrates the nitrosation potential. McIntyre and Scanlon (1993) used a much higher concentration of nitrite to demonstrate that the limiting factor in nitrosamine formation in several foods was not the availability of the amine precursors, but the amount of nitrosating agent. This was also true in our case when extracts from the unused nettings were nitrosated in the same fashion. Here, we saw an increase in NDBzA values in 7 of the 8 samples nitrosated, going from a mean of 71.9 ppb to a mean of 3406 ppb. The nitrosamine results obtained from both the hams and nettings after nitrosation clearly shows the dependence on added nitrite, even though no correlation was previously found between NDBA or NDBzA on the exterior ham surface and residual nitrite (Pensabene *et al.* 1992). However, there still may be an association between ingoing nitrite and the nitrosamine, as first reported for the formation of NPYR in fried bacon by Sen *et al.* (1974). For this reason, we processed netted hams with both the permitted ingoing level of 200 ppm sodium nitrite and with a significantly lower level, 100 ppm. This level of reduced nitrite was selected for investigation since Ingram (1974) considered at least 100 ppm necessary to retain protection against *Clostridium botulinum*. The results are shown in Table 1. A highly significant ($p < 0.01$) mean reduction of 31% in NDBzA was noted in the outer portion of the ham, when the ham was processed with 100 ppm sodium nitrite. There was no significant ($p < 0.05$) difference in NDBzA between the nettings or between the cross-sectional slices processed at the 100 ppm and 200 ppm nitrite levels. Various

TABLE 1.
N-NITROSODIBENZYLAMINE IN HAMS PROCESSED WITH 100 VS 200 PPM SODIUM NITRITE

	N-nitrosodibenzylamine, ppb			
	Range	Mean	Range	Mean
$NaNO_2$ Added	200 ppm	(n=12)	100 ppm	(n=14)
Ham (surface)	76.9-379.1	166.6	78.5-170.7	114.8
Ham (slice)[a]	3.5-15.5	8.6	3.3-11.8	7.0
	125.1-332.9	203.5	15.3-239.5	173.8

[a] Cross-sectional slice

correlations for NDBzA were examined with the data split between the 100 and 200 ppm nitrite levels. For the 200 ppm nitrite samples, the outer surface NDBzA values were highly correlated with the netting and slice values; no such correlation was observed for the 100 ppm nitrite-prepared samples. Again, this suggested that the amount of nitrosating agent, either in the form of nitrite or nitrogen oxides, is a principal factor in the amount of nitrosamine formation. It also indicated that there is sufficient precursor available for additional nitrosamine formation. While this approach of reducing the amount of nitrosamines by decreasing the amount of ingoing nitrite may be an effective one, it cannot be recommended at this time. Additional research is needed since nitrite performs a number of important functions when added to meat. It causes flavor, color, and textural changes that are characteristic of cured meat products (Rubin 1977). In addition, nitrite imparts or has antioxidant and anti-microbial properties. The latter is especially important in preventing the outgrowth of the pathogen, *C. botulinum*. Therefore, before any action is taken on nitrite reduction, an assessment needs to be made on the keeping qualities and microbial integrity of products made with lower levels of nitrite than are currently employed.

Instead of taking a processing approach to reduce the nitrosamine content of netted boneless hams, we decided to pursue a different one that would be much easier to implement. Using limited data, Sen *et al.* (1988) was unable to show that removing the nettings immediately after processing or placing a collagen barrier between the nettings and meat was effective in significantly reducing the interaction between the rubber components and nitrite at the product surface. These authors and other workers did not investigate the effect of leaving the nettings on the hams, post-processing, for an extended time period. It is common practice among processors to produce and keep hams in storage, at just below freezing, to meet peak holiday demands for this product. Some of them leave the nettings on the hams, until the time of sale, to give them more visual appeal, the so-called "country ham" look. These hams can also be stored, in contact with the nettings, under refrigeration conditions in a display case prior to their purchase. For this reason, we conducted a series of experiments with the nettings left on to determine whether this practice would have any effect on the nitrosamine content on the ham surface. In the first experiment, boneless hams were prepared, then stored at -2.2C for up to 12 weeks. The exterior surface of the hams and their nettings were analyzed every 4 weeks. The NDBzA results are shown in Fig. 1. For both the outer ham surface and the nettings, highly significant ($p < 0.01$) differences in the NDBzA levels were found among the samples, as expected, and among the times of storage. The NDBzA on the outer surface of the hams increased from 147 ppb to 318 ppb after the 8 week period, with the nitrosamine nearly doubling in the 4 to 8 week interval. A

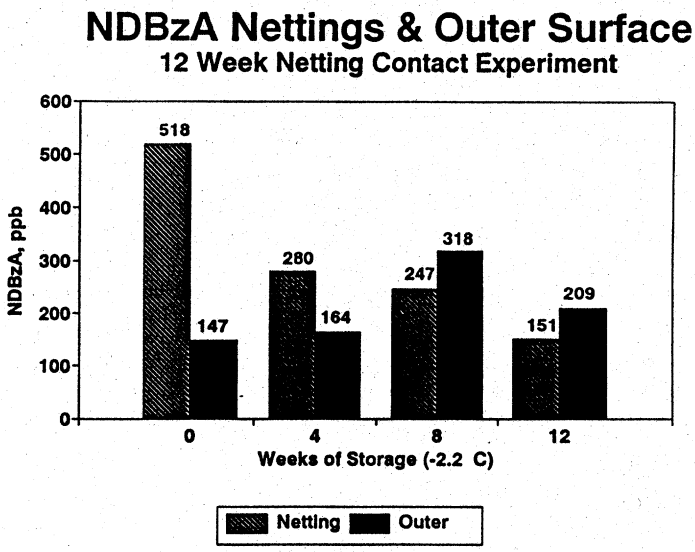


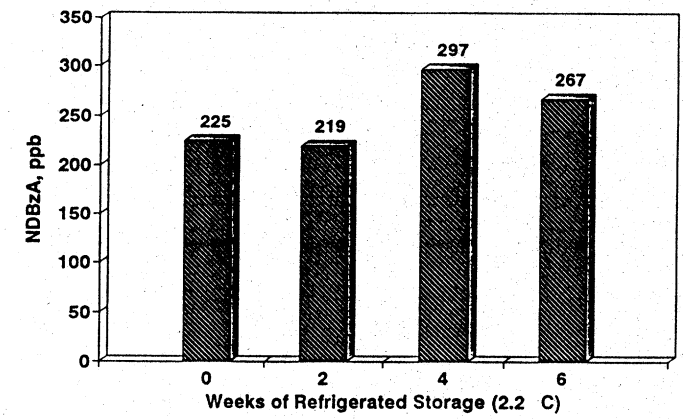
FIG. 1. HAMS WITH NETTINGS LEFT ON UP TO 12 WEEKS: NDBZA IN NETTINGS AND ON THE OUTER SURFACE

decline was noted from 8 to 12 weeks to 209 ppb NDBzA. Examination of the nettings, removed at the same time intervals, showed a decline from 518 to 151 ppb NDBzA over the same 12-week period. Here the maximum decline occurred between the zero and 4-week period.

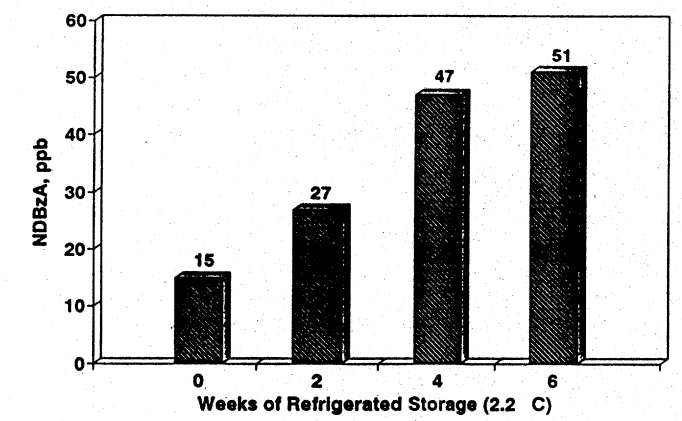
In the second experiment, twelve hams were refrigerated at 2.2C for up to 6 weeks, the nettings removed at 2-week intervals and the outermost 0.64 cm taken for analysis. The overall mean for NDBzA was 252 ppb. The results are shown in Fig. 2a. The NDBzA in the samples at zero time contained a mean value of 225 ppb. This stayed fairly constant with a mean of 219 ppb through the second week. There was a significant increase to 297 ppb NDBzA after 4 weeks and a significant decline to 267 ppb after 6 weeks. This appears to indicate that additional nitrosamine is forming on the ham surface or being transferred from the netting after 2 weeks. Analysis of variance (ANOVA) of the data obtained from 8 samples at a depth of 0.64 cm below the exterior surface, also showed highly significant ($p < 0.01$) differences in NDBzA level among the different storage time intervals. Figure 2b shows an increase from 15 ppb at zero time to 51 ppb at 6 weeks. This

appeared to be a simple penetration from either the surface or from the netting into the ham. Examination of 12 used netting samples at 0, 2, 4 and 6 weeks, which were exposed to the ham surface, showed highly significant differences among samples and exposure times. The results are shown in Fig. 2c. Here the nettings from the 0 week storage gave the least NDBzA and the 2 week storage the greatest, with the 4 and 6 week nettings having lower values not significantly different from each other. These results indicate that additional NDBzA is formed in the netting up to about 2 weeks, then declines. This decline may be due to transfer of the nitrosamine into the product or normal depletion of the nitrosamine. The results suggest that there are multiple processes occurring including additional nitrosamine formation in the netting and/or on the product surface, and some decline of nitrosamine level with time. Regression analysis showed there was a highly significant correlation between the nitrosamine levels in the outer surface and the netting levels. Regression analysis performed on all the data, over all the time intervals indicated that the netting was a major factor in the amount of NDBzA detected. No correlation was found in NDBzA values between the surface and 0.64 cm depth beneath the surface or between the latter and the netting values. Analysis of both the exterior surface and nettings at "zero time" could not demonstrate that additional nitrosamine formed, since a direct comparison was not possible between samples where the netting was left on or taken off for the same amount of time. Therefore, hams were prepared and sampled where both "net off" and "net on" portions were refrigerated for the same period of time prior to analysis.

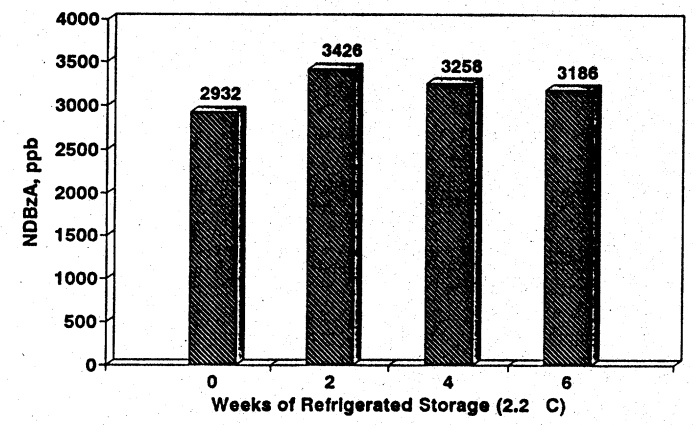
In this third experiment, six hams were stored under the same refrigeration conditions, 2.2C, but this time some of the nettings were removed at zero time in order to make a direct comparison with the samples stored with the nettings still on. Both the outer surface and next 0.64 cm depth portion were analyzed for both NDBzA and its precursor amine, DBzA. As noted previously, ANOVA of the overall outer surface NDBzA values showed highly significant ($p < 0.01$) differences among the samples, times of storage and between on/off types. Duncan's test of differences between individual type means showed a significant ($p < 0.05$) difference between the ham outer surface ($n=48$) values for those where the netting was left on versus those taken off, 303 vs 271 ppb NDBzA. This indicates that continued exposure of the ham to the netting results in increased NDBzA being present on the outer surface. Figure 3a shows the mean values for the outer surface of 12 ham samples where the nettings were left on over the 6 week period. A significant, but modest decline in NDBzA was noted from the 0 to 4 week period (303 to 266 ppb). At the 6 week, there was a mean increase of approximately 90 ppb to 357 ppb NDBzA, above the 0 week level. This suggested that competing processes were taking place, i.e., the



A) Outer Layer NDBzA Netting Left on the Ham



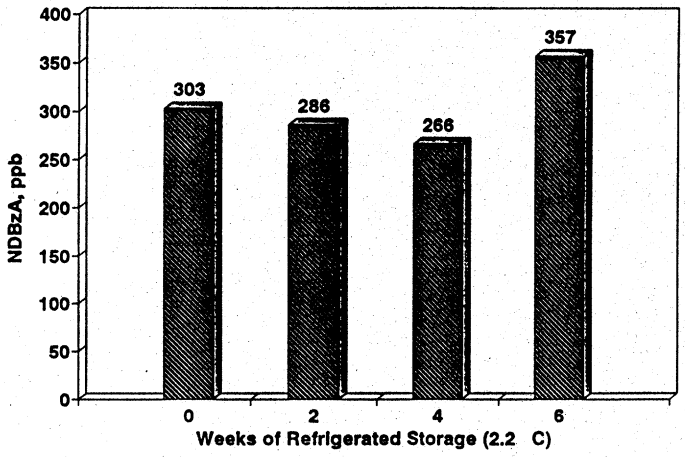
B) Inner Layer NDBzA Netting Left on the Ham



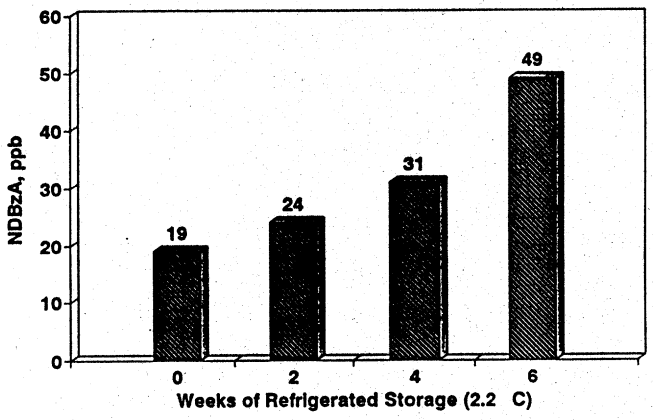
C) Netting Layer NDBzA Netting Left on the Ham

FIG. 2. SIX WEEK HAM STORAGE EXPERIMENT, NDBZA VALUES; (NETTINGS REMOVED AT THE SAME TIME AS THE HAMS.) A) OUTER SURFACE; B) INNER LAYER; C) NETTING

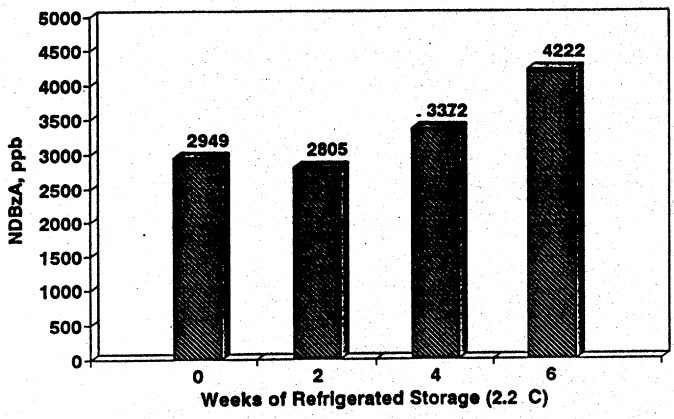
normal depletion of the nitrosamine on the product surface and additional formation of NDBzA on the product surface or netting and transfer to the surface. Over the extended period of 6 weeks, the latter seemed like the dominant mechanism. To test this, NDBzA data was obtained for the ham samples 0.64 cm below the surface. Although lower than the surface values, even here the continued indirect exposure of the ham samples to netting contact resulted in significantly higher levels of NDBzA than those where the nettings were removed, 30.7 vs 23.6 ppb (n=48) NDBzA. While several processes may be taking place on the outer surface, as suggested in earlier experiments, the nitrosamine results shown in Fig. 3b, suggest that beneath the outer surface, diffusion of the nitrosamine from the outer surface into the ham is the dominant one. Examination of the outer surface samples (n=12) for DBzA showed that after initial transfer from the netting, the amine decreased from 710 ppb to a plateau of 330 to 390 ppb during the 2-6 week period. The amount of DBzA was approximately ten times the level of nitrosamine found on the ham surface. This level of amine is large enough to serve as a reservoir for nitrosamine formation, without further transfer of amine from the netting. Additional information was obtained from the analysis of the nettings removed from the hams during the same storage intervals. A highly significant difference ($p < 0.01$) was found in the mean NDBzA values for the nettings (n=48) left on the hams for the various time intervals versus those that were taken off (3337 vs 2724 ppb). These results also confirmed that increased contact time with nitrite-containing meat leads to increased NDBzA levels on the ham outer surface. As shown in Fig. 3c, the nettings (n=12) left on the corresponding hams for the same time intervals, gave lower, but not significantly different NDBzA values at the 2 weeks storage time than at the 0 weeks; they were 2949 and 2805 ppb NDBzA, respectively. At 4 and 6 weeks there was marked increases in NDBzA content to 3372 and 4222 ppb, respectively. These results show that additional nitrosamine forms in the netting with contact time, and then becomes available for transfer and migration into the product. Examination of the 12 nettings, removed from the hams showed a decline in NDBzA from 2949 to 2353 ppb over the 0 to 6 week period. However, the 4 week sample (mean 2930 ppb) was not significantly different from the zero time with an initial decline at two weeks. This finding helps explain the results found in the outer surface samples. For whatever reason the netting values become higher, as was the case for the 4 week nettings, so do the surface values. Overall, highly significant ($p < 0.01$) correlations were found between the inner ham NDBzA and the: (1) weeks of storage, (2) netting NDBzA and (3) outer surface NDBzA. These reflect the simple migration that is occurring. The inner 0.64 cm NDBzA concentration was also significantly ($p < 0.05$) correlated with the outer DBzA concentration. Significant correlations



A) Outer Surface NDBzA Netting Left on the Ham



B) Inner Layer NDBzA Netting Left on the Ham



C) Netting NDBzA Netting Left on the Ham

FIG. 3. SIX WEEK ON/OFF STORAGE EXPERIMENT, NDBZA VALUES; (NETTINGS REMOVED BOTH AT 0 TIME AT 2,4 AND 6 WEEKS.) A) OUTER SURFACE; B) INNER LAYER; C) NETTING

were not obtained for the outer surface NDBzA and other factors, except for outer DBzA concentration ($p < 0.01$), which was found in our other study (Pensabene *et al.* 1995). They probably were not observed because of the more complicated reactions and interactions taking place on the product surface. While these correlations were statistically significant, the r^2 values were generally low, since most of these relationships are obviously not linear.

The overall results strongly suggest that removal of the netting post-processing would help to reduce the amount of nitrosamine in the boneless hams processed in elastic rubber nettings. In addition, a change in the rubber formulation that would reduce or eliminate the amount of precursor would also be highly advised.

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